Characterization of *Actinomyces naeslundii* Type 1 Fimbria by LC-MS/MS

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Objectives

- **To characterize the fimbria of the gram-positive bacterium *Actinomyces naeslundii***

Introduction

The **adhesion of bacteria** to host tissue surfaces is commonly **mediated by** proteinaceous thread-like structures referred to as **fimbriae**.[1-3] Such structures isolated from gram-negative bacteria are generally composed of different major and minor fimbrial subunits and maintained by non-covalent interactions between adjacent proteins. Fimbriae also occur on certain **gram-positive bacteria** such as ***Actinomyces naeslundii***, a microorganism that colonizes the human **oral cavity**.[4] Biogenesis of these fimbriae depends on a **novel pathway** and may involve the formation of covalent bridges between fimbrial subunits.[2] To further examine this possibility, purified *A. naeslundii* type 1 fimbriae have now been subjected to **chemical and enzymatic cleavage** and the products **characterized by LC-MS/MS analysis**.

Methods

Fimbriae were **removed** from ***A. naeslundii*** T14V by **sonification** of washed bacteria and isolated in fractions collected at the void volume of an Agarose 5M gel filtration column. Type 1 fimbriae were precipitated in the presence of 20% saturated ammonium sulfate, dissolved in Tris-buffered saline and passed through an immunoaffinity column to remove type 2 fimbriae.[4] **Purified type 1 fimbriae**, which appeared as thread-like structures by electron microscopy, were subjected to a variety of **chemical and enzymatic cleavages**, including dilute acid hydrolysis, trypsin and pepsin digestion. The resulting fragments were **characterized by LC-MS/MS** using a Micromass QTOF2 mass spectrometer and further identified by *de novo* peptide sequencing using the MassLynx Software.

Conditions of LC-MS:

Mobile phase:

Solvent A: 0.2% formic acid

Solvent B: ACN containing 0.2% formic acid

Stationary phase:

Zorbax C18 SBW column, 0.15 µm x 10 cm

Sample size: ca. 500 fmol

Gradient: 10-90% solvent B

Flow rate: 10 µL/min, 1:10 split

Temperature: ambient

Fig. 1: Instrumentation: Micromass CapLC and QTOF2.

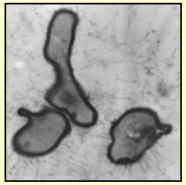


Fig. 2: Electron micrograph of a thin section of *Actinomyces naeslundii* showing the presence of cell surface fimbriae on this microorganism.

Fig. 3: SDS-PAGE of *Actinomyces naeslundii* T14V type 1 fimbriae (lane1) and Western immunoblot showing the reaction of a specific monoclonal antibody with native type 1 fimbriae (lane 2) and the cloned fimbrial subunit (lane 3).[4]

Results

Actinomyces naeslundii **fimbriae** were **digested** with a combination of chemical and enzymatic cleavages and investigated by LC-MS/MS. **Dilute acid hydrolysis**, which cleaves at the amino- and carboxy-terminal site of **Asp**, was found to be particularly effective, even though it yielded very complex peptide maps that needed extensive manual evaluation. **Complexity** was even **increased** when **followed** by a **tryptic digest**. **Pepsin** was the only enzyme that was able to digest the fimbriae without prior dilute acid hydrolysis. The combination of different cleavage methods has currently allowed us to **cover more than 95%** of **FimP**, the major **subunit** of A. naeslundii T14V type 1 fimbriae (Fig. 4). **FimP** is a 57 kDa protein composed of **503 amino acid** residues.[5] The last 38 residues, beginning with Leu at position 466, form a cell wall anchoring motif (LPLTGA) that is typically found in gram-positive bacteria.

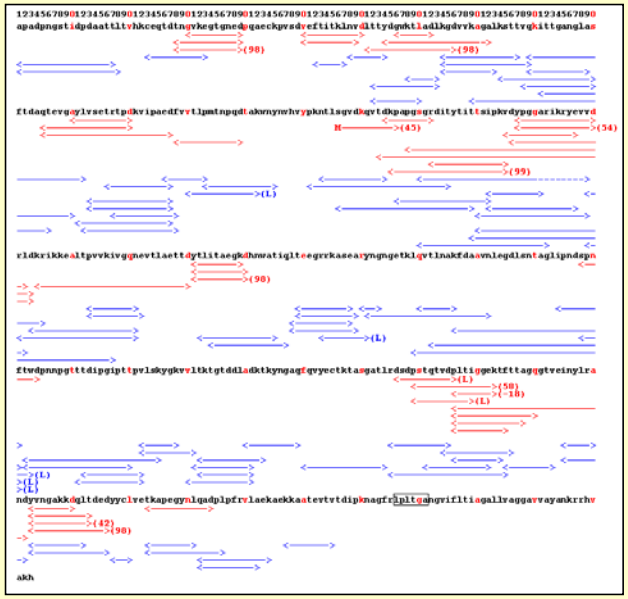
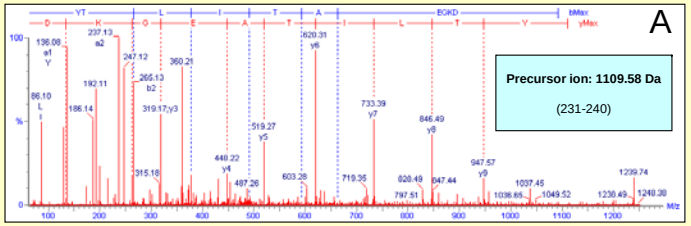


Fig. 4. Sequence and sequence coverage of Actinomyces T14V type 1 fimbrial subunit. Dilute acid/trypsin fragments are given in red and pepsin fragments in blue. Modified/linked peptide fragments are indicated by the numbers/"L"-label in brackets.

Careful investigation of the MS/MS data also revealed a **variety** of **modified** and **linked** cleavage fragments. Most notably, a **+98 Da** addition is seen in **many fragments**, e.g. 28-39, 231-240, 403-416 and others. In addition, **other modifications** (+42 Da, presumably an acetylation; +45 Da, unknown; +54 Da, unknown) were found.



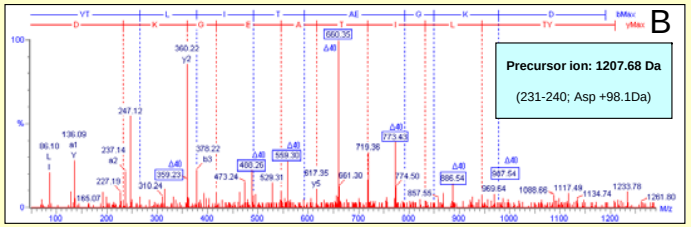


Fig. 5. De-novo sequencing of dilute acid hydrolytic unmodified (A) and modified (B) fragment 231-240. The modification clearly shows a 98.1 Da addition, which gives a typical Δ 40 Da and Δ 58 Da fragmentation pattern.

Extensive evaluation of the MS/MS data allowed the **unambiguous identification** of the location of the **+98 Da modification**. As apparent from Fig. 5, the +98 Da modification is located at the **aspartic acid** residue. The observed typical Δ 40 and Δ 58 fragmentation pattern might aid its molecular identification.

Conclusions

First study of *A. naeslundii* **fimbriae** showed **crosslinkes** and **considerable post-translational modifications** of the fimbrial subunit.

The so far undescribed +98 Da modification is located at the aspartic acids.

Further studies will be directed towards the **connectivity** of the **fragments** and the **structural characterization of the modifications.**

References

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